The anti-angiogenic agent, E7820, induces changes in the architecture of lymphatic vessels around tumors

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Abstract : Anti-angiogenic agents exert strong inhibitory effects on tumors, and their inhibitory effects on metastasis to regional lymph nodes have also been reported. We confirmed the inhibitory effects of an anti-angiogenic agent, E7820 (provided by Eizai Co., Ltd., Japan), on the growth of VX 2 cancer in a rabbit tongue. We examined the changes in the lymphatic architecture around the tumor. E7820 (0.1ml of 10 mg/ml) was injected into the center of a tongue tumor in rabbits every other day for a total of 4 injections starting at 3 days after the transplantation of VX 2 cancer cells. One day after the last injection, the rabbits were sacrificed. The tongue was excised, and serial frozen sections were made. The sections were stained with 5'-Nase, and their 2-dimensional images were fed to a computer. The lymphatic structure was extracted on the computer, and its 3-dimensional image was constructed and examined. 5'-Nase-positive lymphatic vessels were abolished in the region 200μm around the border of the tumor, and no genesis of the lymphatic capillaries was noted. The growth in tumor volume was inhibited to one-fourth that of controls, and metastasis to the lymph nodes was limited to one-fifth of the control.

Key words : lymphatic vessel, anti-angiogenic agents, integrin α_2, cancer, metastasis

Introduction

The growth of solid tumors has been found to depend on blood vessels that are newly generated by growth factors originating from the tumor cells[1], and drugs have been developed that exert antitumor effects by blocking angiogenesis[2-6]. Anti-angiogenic agents show strong inhibitory effects on tumor growth, and their inhibitory effects on metastasis to the regional lymph nodes have been reported[7]. Understanding the mechanisms of inhibition of metastasis to the lymph nodes through the inhibition of angiogenesis is difficult. However, examining the effects of

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these agents on the other kind of vessels, i. e. the lymphatic vessels, has yielded clues. On the basis of their morphological study, Seki et al. inferred that the inhibition of lymphangiogenesis around tumors induced by TNP-470 inhibited metastasis[8]. The mechanisms of inhibition of angiogenesis by TNP-470 have yet to be clarified, as are the mechanisms of inhibition of metastasis to the lymph nodes. The anti-angiogenic agent, E7820[9], is a new agent that inhibits vascular formation by inhibiting the expression of a membrane surface protein integrin α_2. E7820 was developed by the Eizai Co., Ltd. as an agent for per os administration. Most anticancer agents, including antiangiogenic agents, are administered by intravenous injection in high concentrations or per os in large amounts to raise the locally effective concentration to sufficient levels. Accordingly, there is a high probability of inducing various side effects. However, by injecting E7820 directly into the center of the tumor mass, a high concentration can be obtained locally and a low concentration can be obtained systemically[10].

In this study, we investigated the changes in the lymphatic architecture around tumors, the growth of tumors and the inhibition of metastasis to the lymph nodes induced by the local injection of E7820. For the collecting lymphatic vessels, we used the terminology established by Fujimura et al., who reported detailed studies of these structures[11, 12].

Materials and Methods

1. Materials

1) Experimental animals

We used Japanese white male rabbits (Tohoku Kagaku Yakuhin, Japan) weighing 2.5 kg. The animals were maintained in the vivarium of Iwate Medical University (room temperature : 22±2℃, humidity : 55±5%) for more than one week. We used the animals after confirming that they had no abnormalities and were in good condition. They ingested water and solid foods, RC 4 (Oriental, Japan), ad libitum. The experimental protocol of this study was approved by the Ethics Committee of Iwate Medical University School of Dentistry.

2) Experimental tumors

As experimental tumors, we used VX 2 cancer cells, which are implantable in the rabbit's tongue and readily metastatic to the lymph nodes draining the implanted region[13]. VX 2 cancer is a nodose cancer with intracellular keratin, and histologically resembles human squamous cell carcinoma. The VX 2 cancer cells used in this study were extracted from the tumors of a rabbit bearing VX 2 cancer purchased from Japan SLC. The VX 2 cancer cell line was maintained by a successive transplantation to the thigh muscles of rabbits.

3) Anti-angiogenic agent

The anti-angiogenic agent, E7820, was used in this study, and was provided by the Eizai Co., Ltd. To determine the dose of E 7820 to be applied, preliminary in vivo experiments were conducted using 0.1 mg/ml, 1 mg/ml and 10 mg/ml, which were determined on the basis of results obtained from experiments using a culture system[9]. The concentration of 10 mg/ml showed anti-tumor effects in the preliminary experiment, and this concentration was used in this study. Fifty mg of E7820 was dissolved in a mixture of Dimethyl Sulfoxide (DMSO) (0.35 ml) and Tween 80 (0.65 ml). The E7820 solution was then diluted to 10 mg/ml with a 5% glucose solu-

tion, and this adjusted solution was used for the experiments.

2. Methods

1) Preparation of the VX 2 tongue cancer model in rabbits

The VX 2 tongue cancer model in rabbits was produced by transplanting 0.05 ml of a solution containing approximately 5 x 10^5 VX 2 cancer cells into the muscular layer at 3 mm deep to the surface of the tongue at a site distant from the apex by one third of the rostrocaudal length of the tongue on the left side. The cells were injected under general anesthesia by intravenous injection of 0.5 mg/kg of pentobarbital sodium (Nembutal, Abbot Laboratories, USA). Tumors with a diameter exceeding 4 mm were used for the experiments.

2) Control group

(1) Five rabbits were used in the following experiments. To observe the size of the tongue tumor, the metastasis to the lymph nodes and the muscular tissue around the tumor in the animals to which E7820 was not administered, the size of tumors was measured every other day from the day when the long diameter of the tumor exceeded 4 mm. The animals were sacrificed 7 days after completion of the measurement. To study the metastasis of VX 2 tongue cancer to the lymph nodes that drained the region of administration of the agent, the deep cervical lymph nodes were excised. The samples were embedded in paraffin. Hematoxylin-Eosin (H-E)-stained sections were produced from all of the lymph nodes, and the sections were examined histologically.

(2) To observe the lymphatic architecture surrounding the locus of administration of the agent, a single injection of 0.1 ml of E7820 (10 mg/ml) was administered into the tongue of normal rabbits. The treated rabbits were sacrificed on the next day. In addition, to observe the changes in the lymphatic architecture induced by the pressure caused by local injection of the agent, a single injection of 0.1 ml of a 5% glucose solution was administered into the tongue of normal rabbits, which were sacrificed on the following day.

3) Experimental group

Five rabbits were used in the following experiments. To observe the lymphatic vessels surrounding the tumor after administration of the anti-angiogenic agent, 0.1 ml of E 7820 (10 mg/ml) was injected into the center of the tumor with 26 gauge injection needles every other day after the long diameter of the tumor exceeded 4 mm. Injections were performed 4 times in total. To estimate the growth of the tumor, we measured its size using a slide caliper, and calculated the tumor volume (mm^3) using the formula of $\{(\text{long diameter}) \times (\text{short diameter})^2\}/2$. In addition, the deep cervical lymph nodes were excised. H-E-stained sections were produced from these lymph nodes and they were examined histologically.

After the rabbit was killed with an overdose of pentobarbital sodium, the tongue was excised. The tissue was fixed in a cold fixative solution of 4 % formaldehyde with 1 % calcium chloride for 30 min. After fixation, the tongue was transected on the frontal plane at the center of the tumor, and the posterior half was used for three-dimensional reconstruction of the lymphatic vessels around the tumor.

4) Preparation of serial sections

After fixation, the tongue was rinsed in 0.1 M Tris-maleate (pH 7.2, 4℃), and frozen in

hexane (−80℃) cooled by dry ice. For three-dimensional reconstruction of the lymphatic vessels, one hundred 10 μm serial frozen sections were cut. We prepared the thin sections of the rabbit's tongue after the method of Kawamoto et al.[14], using the adhesive film transfer kit (Finetec, Japan) and a cryostat (Bright Instrument Co., Ltd., UK).

5) Identification of lymphatic vessels

The frozen serial sections were rinsed in 0.1 M Tris-maleate buffer (pH 7.2, 4℃), and were incubated for 30 min at 37℃ in a 5'-Nase substrate solution of the same composition as Kato's reactive solution[15]. The sections were then rinsed in buffer and immersed in 1 % ammonium sulfide solution for 2 min for chromogenesis. After rinsing in distilled water, the sections were mounted with glycerol and examined histologically.

6) Three-dimensional reconstruction technique

The serial sections stained with 5'-Nase were photographed with a light microscope (E1000M, Nikon, Japan) and a cooled CCD camera (C5810, Hamamatsu Photonics, Japan), and two-dimensional images were fed directly into a computer (XPST550, Dell, USA). Using image processing software (Photoshop ver.5.5, Adobe, USA), we extracted the 5'-Nase stain-positive lymphatic vessels from these images, coordinated the axes of the images and made the threshold treatment. Then, using three-dimensional analyzing software (Voxblast, ver. 3.1, Vaytek, USA), we obtained three-dimensional reconstruction images of the lymphatic vessels with the volume rendering method[16]. By revolving the three-dimensional images, we observed the lymphatic architecture around the tumor in the tongue muscle layer, and compared the lymphatic structure between the control and experimental groups.

7) Statistical analysis

The measured volume of tumors was analyzed with statistical software (InStat 2.03), and the significance of differences in the results were compared between the control and E7820-administered groups by a nonparametric method (Dunn's multiple comparison test).

Results

1. Systemic effects of administration of the agent

No changes in the body weight of the rabbits were found after the administration of E7820. No major side effects, such as depilation and anorexia, were observed with the dose used in this study.

2. Growth of the tumor and metastasis to the lymph nodes

The volume of tumor at the time of the last administration of E7820 was one-fourth that of the control group, which did not receive E7820. Thus, the growth of the tumor was markedly reduced (Fig. 1). The surface of the mucous membrane over the tumor region was smooth, and no ulcer was formed. The border of tumor was sharp. Metastasis to the deep cervical lymph nodes was found in 5/5 control animals that did not receive E7820, while no metastasis was found in 4/5 animals that received the agent (Fig. 2). In the one animal showing metastasis to the lymph nodes, the metastasis size was comparable to the normal ones. Although H-E staining confirmed that cancer cells were localized in the transitional zone from the peripheral to intermediate sinus, the tumor had not proliferated from there or the proliferation

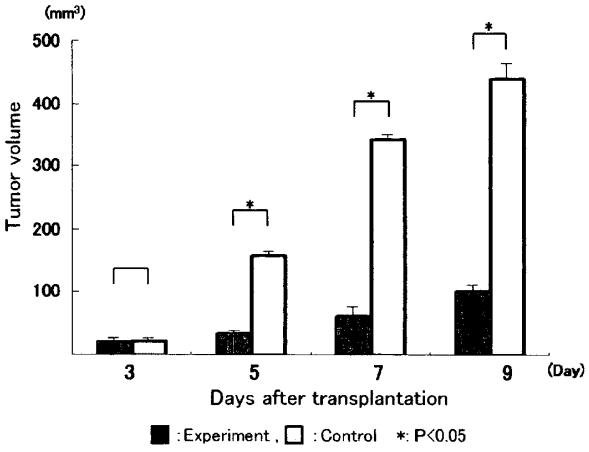

Fig. 1. Volume of the tumor.
In the control group to which E7820 is not administered, the average volume of the tumor is 450 ㎣ on the 9 th day after transplantation. In the group to which E 7820 is administered, the average volume of the tumor is 100 ㎣. The tumor size in the treated animals is reduced to almost one-fourth of that of the control animals.

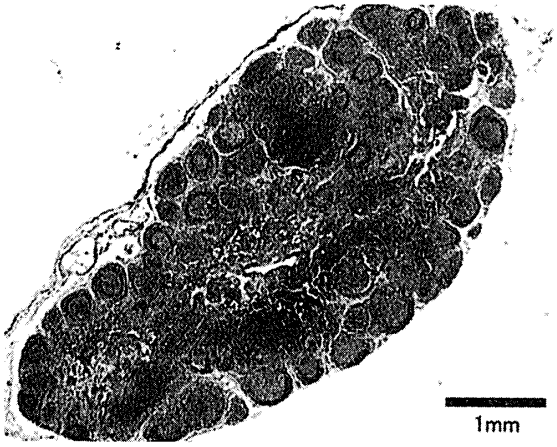

Fig. 2. Lymph node from an E7820-administered animal in which no metastasis is found.

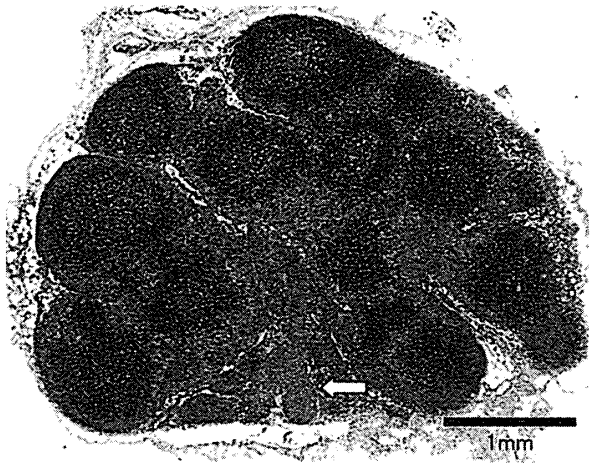

Fig. 3. Lymph node from an E7820-administered animal in which metastasis is found. Arrow indicates the tumor.
Metastasis is found in 1/5 E7820-administ ered animals, but the volume of the nodes is almost one-third of that of the control group and almost the same as that in the normal group.

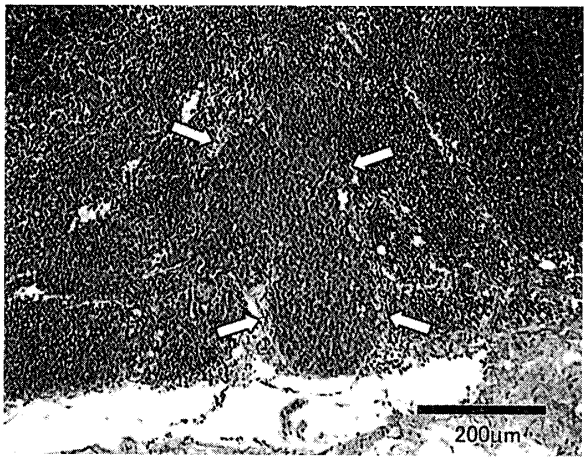

Fig. 4. Higher magnification of Figure 3. Arrows indicate the tumor.
Cancer cells are localized in the peripheral sinus. The cancer cells are viable, but not proliferating.

had been inhibited (Figs. 3 and 4). There was no difference in either the volume of the tumor or the three-dimensional lymphatic architecture between the one rabbit with metastasis to the lymphatic nodes and the remaining 4 animals. Metastasis to the lung was not observed in any of the animals.

3. Histological images and architecture of lymphatic vessels

1) Control group

Comparison between the normal tongue (Fig. 5) and the tongue injected with 5% glucose solution only (Fig. 6) revealed no differences due to the needle injection or to the pressure of injection of the agent solution. No morphological changes were found in any of the lymphatic vessels in the tongue, including the collecting lymphatic vessels below the superior longitudinal muscle (SLCL), the lymphatic vessels

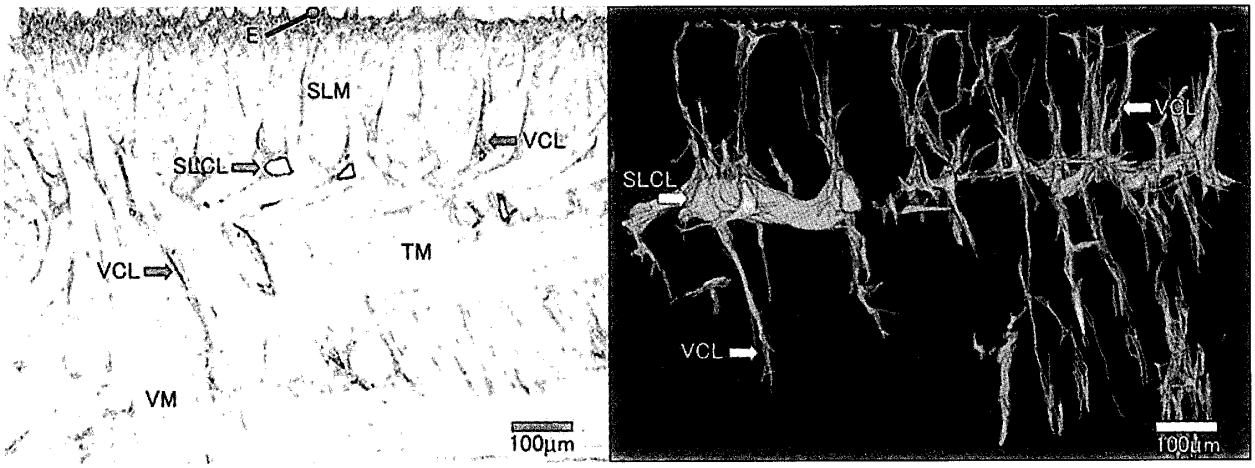

Fig. 5. Lymphatic architecture in the normal tongue.
The lymphatic vessels originating from blind ends join the collecting lymphatic vessels below the superior longitudinal tongue muscle from above and the lymphatic vessels accompanying the vertical tongue muscle originating from the inferior longitudinal muscle from below. Abbreviations: SLCL, collecting lymphatic vessels below superior longitudinal muscle; SLM, superior longitudinal muscle; TM, transverse muscle, VCL, collecting lymphatic vessels accompanying vertical muscle; VM, vertical muscle; E, epithelium.

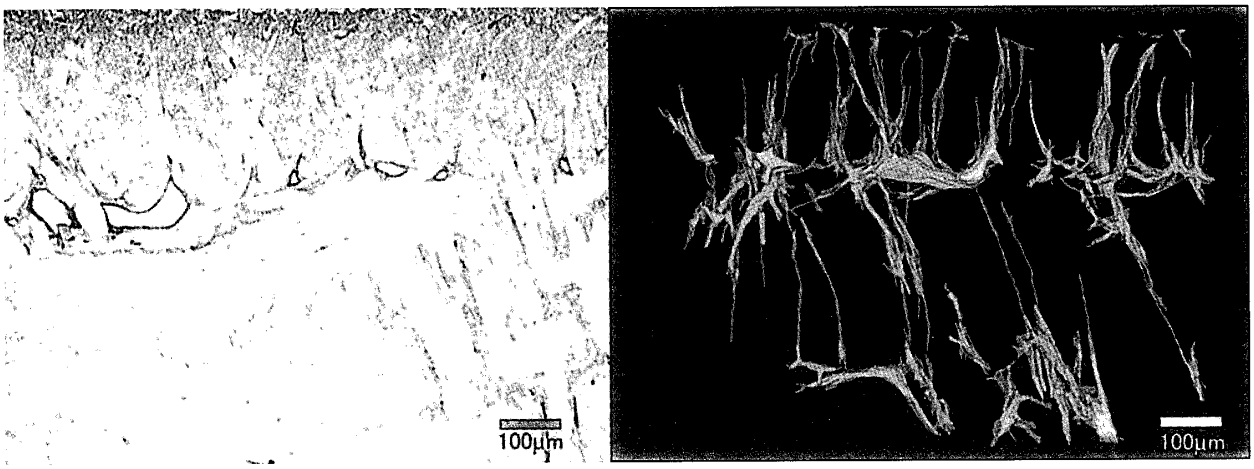

Fig. 6. Lymphatic architecture in a tongue into which 5 % glucose solution is injected.
No differences are found from the normal tongue.

originating from lymphatic capillary network in the lamina propria and joined to the SLCL, and those that accompanied the vertical muscle (VCL) from lower side of superior longitudinal muscle to the SLCL. Similarly, a comparison of the normal tongue with that injected with E7820 (Fig. 7) showed no differences in any of the muscle layers, the SLCL or the lymphatic vessels that originated from lymphatic capillary network in the lamina propria and joined the SLCL. However, in the E7820 -injected animals, the VCL coursing from lower side of superior longitudinal muscle to the SLCL were decreased in number.

2) Experimental group

In the muscular layer around the tumor, the distortion by the pressure caused by the tumor and the changes in form induced by destruction were noticed in the transverse, vertical and longitudinal tongue muscles. However, these changes were very slight compared with that in the control group that did not receive E7820. No changes in the

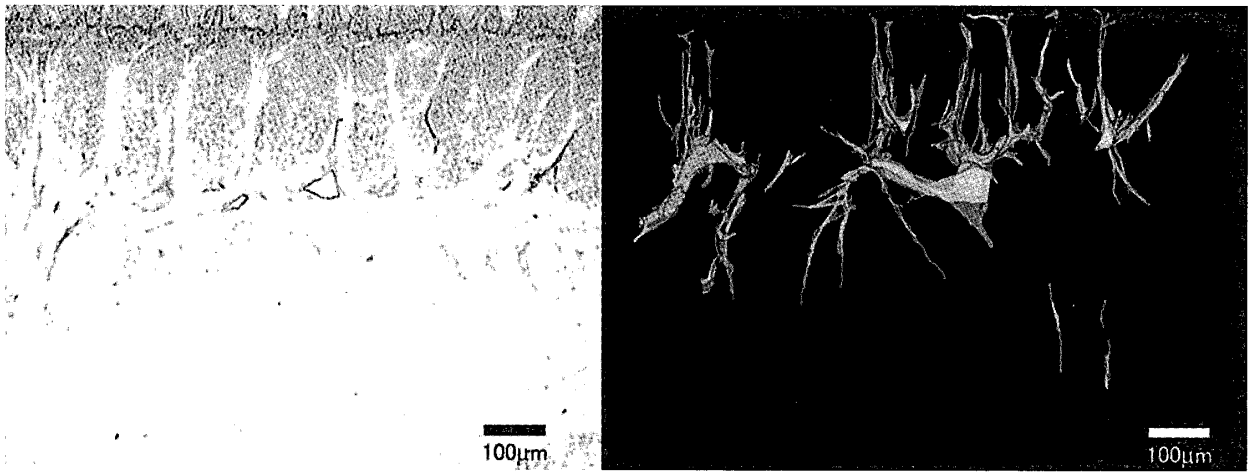

Fig. 7. Lymphatic architecture in a tongue into which E7820 was injected.
No changes are found either in the SLCL or in the lymphatic vessels originating from blind ends and joined to the SLCL. The lymphatic vessels accompanying the vertical tongue muscle from the inferior longitudinal tongue muscle to the SLCL are decreased in number.

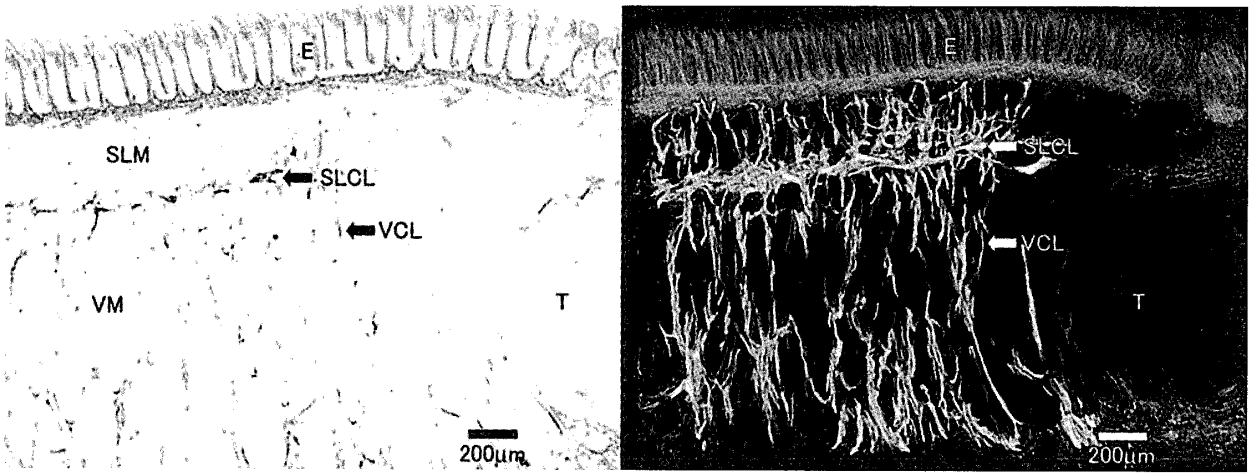

Fig. 8. Lymphatic architecture in a tongue in which an intratumor administration of E7820 is made.
5'-Nase positive collecting lymphatic vessels and lymph capillaries are completely abolished in the region within 200μm around the border of the tumor (T). Angiogenesis of the lymph capillaries was not found. Abbreviations: E, epithelium; the others are the same as in Figure 5.

tissue due to the insertion of the injection needles or the injected agent itself were observed. On the other hand, since the inside of the tumor mass consisted of necrotic tissue, changes in the tissue could not be detected. Within 200 μm around the border of the tumor, 5'-Nase positive collecting lymphatic vessels, all of lymphatic vessels were completely abolished, and angiogenesis of the lymphatic capillaries was not observed. Outside of this region, no changes were found in the number of the lymphatic vessels (Fig. 8).

Discussion

Because of the characteristic property of anti-angiogenic agents to exert anticancer effects not directly on tumor cells, but indirectly by blocking angiogenesis, the period of administration of anti-angiogenic agents is assumed to be longer than anticancer drugs. Accordingly, per os administration is more desirable than intravenous injection, because it is less

invasive to the patients. This is the reason E 7820 was developed for per os administration. Since the long term administration of this agent may cause an accumulation in the body, the systemic effects of E7820 cannot be neglected. Accordingly, for our local cancer therapy, we adopted the method of direct injection of the anti-angiogenic agents into the center of the tumor, to maximize its inhibitory effects on cancer proliferation and metastasis to lymph nodes and to minimize the systemic side effects. We assumed that the influences of pressure on the lymphatic tissue caused by injection of the agent could be neglected, because no effects on the lymphatic architecture in the tissue around the injected site were observed in the group of animals that received a 5 % glucose solution injection into the normal tongue. In the group that received an injection of E7820 the tongue, no effects of the agent were found on the large lymphatic vessels such as the SLCL, in which the turnover of the agent seemed to be slow, but thin collecting lymphatic vessels, such as the VCL to join the SLCL, were disappeared. The loss of these thin collecting lymphatic vessels suggests that this agent may affect not only the newly-generated lymphatic capillaries induced by the cancer cells, but also the activity of the pre-existing lymphatic vessels. The injected agent is expected to diffuse in the normal tongue within a few hours. In the tongue cancer model, however, the diffusion may be delayed due to the presence of necrotic tissue in the center of tumor and the tissue reaction around the tumor. Therefore, its sustained release might be enhanced[17]. As regards to E7820, the speed of diffusion, the concentration in the tissue at the injection site and its influence on pre-existing lymphatic vessels should be examined in future studies.

Anti-angiogenic agents are thought to cause few side effects. Even if they occur, side effects, such as a decrease in body weight, muscular pain and depilation, are much less severe compared with those of anticancer drugs[18]. We obtained similar results after the administration of E7820 every other day for 4 times in total. We did not detect any changes that were assumed to be side effects. The effects of this agent can be assumed to be dose-dependent on the basis of its influences on the tumor, as well as the changes in the lymphatic architecture around the site of injection. Since no side effects were observed, the dose determined in the preliminary experiments is assumed to be appropriate.

Newly generated blood vessels may be required to provide tumors with adequate oxygen and nutrition when the growth of tumors exceeds 1～2 mm[1]. Takeda et al. reported that newly generated blood vessels form a basket-like architecture when the long axis of a tumor of VX 2 cancer exceeded 7 mm[19]. Thus, in the case of VX 2 used in the present study, the advent of newly generated blood vessels was assumed to precede the time when the long diameter of the tumor reached 7 mm. Accordingly we initiated the administration of E7820 when the tumor with a long diameter of 4 mm began to proliferate, i.e., when the advent of newly generated blood vessels was prominent, but the basket-like net of blood vessels was not completed.

Dependence of the growth and metastasis of tumors on newly generated blood vessels has been clarified[1]. Angiostatin was

discovered by Folkman et al.[20]. Since its inhibitory effects on the growth of tumors were reported, attention has been paid to the application of anti-angiogenic agents as a dormant therapy of cancer.

In regards to the mechanisms of action of anti-angiogenic agents, the inhibition of angiogenesis-promoting factors should be mentioned. Two groups of factors are involved in the regulation of angiogenesis. The first is angiogenesis-promoting factors, including VEGF, IL-8 and TP[21, 22, 23]. The second group is the angiogenesis-inhibiting factors, such as angiostatin and endostatin[2, 20]. These promoting- and inhibiting-factors are released not only from cancer cells, but also from the endothelial cells of blood vessels and interstitial cells, such as macrophages[22]. Usually there is a large quantity of the angiogenesis-inhibiting factors in the circulating blood, and, since expression of the inhibiting factors exceeds that of the promoting factors, angiogenesis is inhibited. However, in a tumor and its surrounding tissue, the promoting factors are produced by interstitial cells, such as tumor affiliated macrophages (TAM), and the balance of expression between both factors is lost. It is believed that this leads to angiogenesis in tumors[23]. SU5416[3] and TNP-470[24] are among the angiogenesis-promoting factor-directed agents now at the stage of clinical trials for cancer patients.

On the other hand, the dissolution of the basement membrane of the blood vessels and the extracellular matrix is thought to be indispensable for angiogenesis and deeply involved in the infiltration and metastasis of cancer[25, 26]. The MMP-inhibiting agent blocks angiogenesis by inhibiting the dissolution of the extracellular matrix. Marimastat is one of the representative agents that is at the stage of clinical trials[27]. In addition to these agents, other important factors for angiogenesis and for the infiltration and metastasis of cancer are adhesion molecules, including CD 31, VE cadherin and integrin[28, 29, 30]. Among the integrin family, integrin $\alpha_v\beta_3$ is known to be specifically expressed in the blood vessels generated in metastatic tumors[31]. Among integrin-directed anti-angiogenic agents at the state of clinical trials are an anti-integrin $\alpha_v\beta_3$ antibody, Vitaxin[32], and an integrin α_5 antagonist, EMD121974[33].

E7820 is a new agent whose mechanism of action is relatively clear. E7820 exerts a blocking effect on the formation of the intravascular space by inhibiting the expression of an intercellular adhesion factor, integrin α_2, at the mRNA level[9]. The inhibitory effect of E 7820 on the proliferation of tumor cells was reported in the mouse DAS (dorsal air sack) model in vitro. Integrin decreases with time in the sequence of α_2, α_3, α_5 and β_1 after the administration of E7820. E7820 is thought to produce its effects through various indirect interactions, such as blocking other promoting factors through the inhibition of integrin. We are now investigating these indirect interactions, and are planning to start clinical trials in the near future.

The network of pre-existing lymphatic vessels around a tumor is destroyed by the tumor growth, and lymphatic vessels are newly generated from particular sites of the remaining collecting lymphatic vessels. As this repeatedly occurs, the lymphatic vessels gradually increase the area of contact with tumor cells that have proliferated and

isolated from the tumor. Thus, the likelihood of metastasis is increased. Furthermore, the lymphatic vessels have fewer immune competent cells than blood vessels. Accordingly, once cancer cells reach the lymphatic vessels, it may be more difficult to eliminate them than if they were in the blood vessels, in which there are abundant macrophages and lymph cells[34]. As a result, tumor cells that have invaded the lymphatic vessels would move to the regional lymph nodes. In the regional lymph nodes, immune reactions start before cancer cells flow into the peripheral sinus, and inflammatory cytokines are released from macrophages. Thereafter, cancer cells can be detected in the peripheral sinus[35]. Immediately after this, cancer cells temporarily decrease in number, but they begin to increase in a few hours because the immune responses are lowered. When cancer cells are implanted or immediately after the injection of drugs into tumors, a small amount of cancer cells may be brought to the regional lymph nodes. Since the number of these cancer cells is small, however, it is thought that the proliferation of these cancer cells would occur only rarely due to the cytocidal effects of cytokines[36].

Seki et al. transplanted VX 2 cancer cells into the rabbit tongue, and intravenously injected an anti-angiogenic agent, TNP-470 (30 mg/kg), every other day from 3 days after transplantation for 4 times total. They reported that the volume of the tumor was reduced to one half and that metastasis was inhibited to one-fifth compared with that of control animals[8]. In regards to the lymphatic architecture around the tumor, they confirmed that the lymphatic vessels within 100μm around the border of the tumor were abolished, and that the number of collaterals that arose from the SLCL and extended towards tumor was reduced. They suggested that these results resulted in the inhibition of metastasis to the lymph nodes. The intratumor administration of E7820 reduced the volume of tumor remarkably to one-fourth and inhibited metastasis to the lymph nodes to one-fifth compared with that of the control animals. With respect to the lymphatic architecture around the tumor, the lymphatic capillaries and the collecting lymphatic vessels were completely lost within a region of 200μm around the border of tumor, which is twice as large as the region in the case of TNP-470 administration. Thus, E 7820 widely inhibited angiogenesis of the immature lymphatic capillaries that are induced by cancer cells and readily take up cancer cells in the same way as the newly generated blood capillaries. This inhibition may have lowered the probability of cancer cells to reach the initial lymphatic vessels and, thus, inhibited metastasis. In the present study, metastasis to the lymph nodes was found in only one animal after application of E7820. This result was similar to that obtained by the administration of TNP-470. Viable cancer cells were localized in the transitional zone from the peripheral to the intermediate sinus, but these cells had not proliferated. In contrast to anticancer agents that are directed at the cancer cells themselves, anti-angiogenic agents are thought to exert no direct cytocidal effects on cancer cells[37]. Since the proliferation of cancer cells in the metastasized lymph nodes was inhibited by E7820, however, this anti-angiogenic agent may exert a certain direct effect on cancer cells. The dose of administration of E7820 in

the present study was set to a local injection of 100μl of 10mg/ml solution, which corresponded to 0.5mg/kg of body weight of rabbits. In experiments in which E7820 was administered to nude mice that had received a transplantation of Lovo cells, the administration of 100mg/kg of E7820 twice a day was required to reduce the volume of the tumor to one-third[9]. This finding indicates that the dose used in the present experiments was very small, and suggests that the concentration in the blood was also very low.

The results of this study suggest that with the minimum dose of administration we obtained the maximum effects with respect to the inhibition of tumor growth, as well as the inhibition of metastasis to the lymph nodes by abolishing the lymphatic vessels. These results also confirm the effectiveness of this agent and evaluate the validity of the intratumor administration technique for clinical application.

Conclusions

We administered a anti-angiogenic agent, E7820, locally to a VX 2 tongue cancer model in rabbits, and examined its inhibitory effects on the growth of tumors and metastasis to the lymph nodes. We also observed alterations in the three-dimensional lymphatic architecture around the tumors. The following conclusions were obtained.

1. Intratumor administration of E7820 completely abolished the lymphatic capillaries and the collecting lymphatic vessels within the region of 200μm around the border of tumor.
2. Intratumor administration of E7820 inhibited the growth of the tumor to nearly one-fourth and metastasis to the lymphatic nodes to one-fifth of that observed in the control animals.
3. These results suggest that metastasis to the lymph nodes was inhibited by the loss of the lymphatic vessels around the tumor. To the contrary, however, lymphangiogenesis and metastasis to the lymph nodes may have been inhibited by the inhibition of the growth of the tumor.
4. Intratumor administration of E7820 may be useful as a means of future cancer treatments.

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血管新生阻害薬E7820による腫瘍周囲リンパ管の構築変化

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抄録：固形腫瘍の増殖は腫瘍細胞に由来する増殖因子によって新生される血管に依存することが明らかになり，その結果血管の新生を阻害することにより，抗腫瘍効果を期待する薬剤が開発されてきた。本実験で用いたE7820は，血管の管腔形成を抑制する新しい血管新生阻害薬である。一方，癌治療薬は，ほとんどが局所での有効濃度を考慮して高濃度で経静脈的に，または大量を経口的に投与されており，様々な副作用を引き起こす可能性が考えられる。そこでわれわれは薬剤を少量で，しかも高濃度の状況を得るため，腫瘍塊中心部に薬剤を直接注入する投与方法を用いて検索した。

ウサギ舌辺縁部に*VX*2癌細胞を移植し，移植後3日目より隔日で4回，腫瘍塊中心部に*E*7820（10mg/ml）をそれぞれ100μl注入した。最終投与の翌日に屠殺し舌を摘出，凍結包埋し，10μmの連続切片を作成した。切片は5'-*Nase*染色を施し，コンピュータに二次元画像として入力，リンパ管を抽出後，三次元再構築し観察した。またリンパ節転移の有無に関しては，深頸リンパ節を摘出後，連続切片標本に*H-E*染色を施し，病理組織学的に検索した。

腫瘍の体積は，対照群と比較して約1/4であり著明に腫瘍の増殖が抑制された。また対照群では，全例に深頸リンパ節への転移が認められたのに対し，薬剤投与群では，80%に転移が認められなかった。腫瘍周囲のリンパ管は，腫瘍外郭200μmの範囲には，5'-*Nase*陽性のリンパ管が完全に消滅しており，毛細リンパ管の新生は認められなかった。

腫瘍の増殖は本薬剤により抑制されたと考えられた。また薬剤投与により，腫瘍外郭200μmの広範囲のリンパ管が消失したことから，癌細胞がリンパ管源流に到達する確率が低くなり，リンパ節転移が抑制されたと考察した。*E*7820の腫瘍内投与により，少量の薬剤投与量で著効を認めたことから，本薬剤の有効性と，腫瘍内投与による臨床応用の可能性が示唆された。

キーワード：リンパ管，血管新生阻害薬，インテグリンα^2，癌，転移